

## Short Report: High Frequency of Diarrheagenic *Escherichia coli* in Human Immunodeficiency Virus (HIV) Patients with and without Diarrhea in Lima, Perú

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**Abstract.** Diarrhea is still a prevalent health issue in HIV patients. Our objective was to characterize the different diarrheagenic *E. coli* (DEC) groups in stools from adult HIV patients. Cross sectional study: We enrolled HIV-positive and -negative patients with and without diarrhea from a tertiary-care center of Lima, Peru. Clinical data was recorded and a stool sample per patient was cultured. Multiplex PCR was used to detect different DECs. One hundred eighty-four participants were enrolled. The frequency of having at least one DEC was more common in HIV-positive than HIV-negative patients with diarrhea (42% versus 20%,  $P < 0.05$ ). The enterotoxigenic *E. coli* (ETEC) was the most common DEC in patients with diarrhea, 13% in HIV patients. The diffusely adherent *E. coli* (DAEC) was only present in HIV positive patients with diarrhea (10.1%). Different types of DEC are frequent in stools from HIV-positive patients.

Since the highly active anti-retroviral treatment (HAART) was introduced for acquired immunodeficiency syndrome (AIDS), the incidence of opportunistic infections has decreased and new pathogens, such as diarrheagenic *Escherichia coli* (DEC) groups,<sup>1</sup> are now associated with diarrhea in human immunodeficiency virus (HIV) patients. Because antibiotic therapy may be successful in cases where pathogenic bacteria are identified, studies focusing on bacterial diarrhea in AIDS patients are needed. The DECs have been classified into six groups based on specific genes that determine their virulence factors: enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), Shiga toxin producing *E. coli* (STEC), enteroaggregative *E. coli* (EAEC), enteropathogen *E. coli* (EPEC), and diffusely adherent *E. coli* (DAEC).<sup>2</sup> The role of different DECs in causing diarrhea in HIV-infected people in Latin American countries has not been adequately addressed because most studies have not examined for all currently recognized groups of DECs. Our objective was to characterize the different DECs in stools from adult HIV patients in Lima, Perú.

### METHODS

This was a descriptive, cross-sectional study. We enrolled HIV-positive (HIV<sup>+</sup>) patients older than 18 years of age with diarrhea (D<sup>+</sup>) and without (D<sup>-</sup>) at the HIV clinic of Hospital Cayetano Heredia, a referral center in Lima, Perú, during the period 2007–2008. We also enrolled adult HIV-negative (HIV<sup>-</sup>) or unknown HIV status with diarrhea who attended the emergency room and HIV<sup>-</sup> patients without gastrointestinal complaints. Because HIV prevalence in the general population of Perú is low (~0.5%), we analyzed HIV<sup>-</sup>D<sup>+</sup> and HIV-unknown status D<sup>+</sup> in the same group. The HIV status was determined by the review of patient's charts or by performing an enzyme-linked immunosorbent assay (ELISA) test. Patients who received antibiotics (other than trimethoprim-sulfamethoxazole) during the previous 7-day period were not included. Approval was obtained from the Institutional Review Board of the Universidad Peruana Cayetano Heredia

and the Hospital Nacional Cayetano Heredia. Patients were interviewed for clinical data including sex, age, weight, height, gastrointestinal symptoms, and HIV status. The CD4 count and the use of HAART were recorded from patients' charts. Diarrhea was defined as  $\geq 3$  loose or watery stools in a 24-h period. Participants without diarrhea were enrolled if no gastrointestinal symptoms were present during the previous month. One stool sample per patient was cultured. Five *E. coli* colonies were isolated from MacConkey agar plates and analyzed by a multiplex real-time polymerase chain reaction (PCR) method using primers for each type: EAEC (*aggR*), ETEC (*lt*, *st*), EPEC (*eaeA*), STEC (*stx1*, *stx2*), EIEC (*ipaH*) and DAEC (*daaD*) as previously described.<sup>3,4</sup> This PCR has a sensitivity of 99% and a specificity of 100%<sup>3</sup> and has been previously validated in our laboratory.<sup>4</sup> Stools were also analyzed for the detection of *Salmonella*, *Shigella*, *Campylobacter*, and *Vibrio* species. The stool was not analyzed for enteric viruses.

### RESULTS

One hundred eighty-four (184) individuals were enrolled over a period of 2 years (2007–2008); 69 HIV<sup>+</sup>D<sup>+</sup>, 45 HIV<sup>+</sup>D<sup>-</sup>, 40 HIV<sup>-</sup>D<sup>+</sup> (10 HIV<sup>-</sup>, and 30 HIV-unknown status [presumably negative]), and 30 HIV<sup>-</sup>D<sup>-</sup>. Mean age was 35.6 years (SD  $\pm$  11.4). There were no significant differences in age, sex, and body mass index between HIV<sup>+</sup> and HIV<sup>-</sup> patients. Episodes of diarrhea with duration of more than 1 week were more frequent in HIV<sup>+</sup> than HIV<sup>-</sup> patients (60.8% versus 10%,  $P < 0.05$ ). Among HIV<sup>+</sup> patients, those with diarrhea were less likely to be receiving HAART: 39.1% and 60% in HIV<sup>+</sup> D<sup>+</sup> and HIV<sup>+</sup> D<sup>-</sup>, respectively.

The frequency of having at least one DEC was more common in HIV<sup>+</sup>D<sup>+</sup> (42.0%) than in HIV<sup>-</sup>D<sup>+</sup> (20%) ( $P < 0.05$ ) (Table 1). Other bacteria than DEC were found in 1.4% of HIV<sup>+</sup>D<sup>+</sup> patients (one *Salmonella* sp. and one *Shigella flexneri*). No bacteria other than DECs were found in the HIV<sup>-</sup>D<sup>+</sup> group. The frequency of having at least one DEC tended to be higher in HIV<sup>+</sup>D<sup>+</sup> than HIV<sup>+</sup>D<sup>-</sup> (42.0% versus 26.7%). The ETEC was the most common DEC in patients with diarrhea (13% in HIV<sup>+</sup> and 12.5% in HIV<sup>-</sup> patients). The EAEC was more frequent in HIV<sup>+</sup> D<sup>-</sup> than in HIV<sup>+</sup> D<sup>+</sup> patients (11.1% versus 7.2%). The DAEC was only present in HIV<sup>+</sup>D<sup>+</sup> patients (10.1%) (Table 1).

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TABLE 1

Baseline characteristics and diarrheagenic *Escherichia coli* isolated from human immunodeficiency virus (HIV)-positive and -negative patients with and without diarrhea\*

DEC type	HIV-positiven		HIV-negative	
	With diarrhea	Without diarrhea	With diarrhea†	Without diarrhea
Patients, no.	69	45	40	30
Age in years, mean (SD)	34.0 (8.5)	36.5 (9.9)	36.9 (15.7)	36.3 (12.8)
Male sex, no. (%)	54 (78.3)	28 (62.2)	25 (62.5)	18 (60.0)
+BMI, mean (SD)	21.2 (3.7)	23.3 (4.2)	23.3 (4.2)	24.2 (4.2)
Diarrhea episode more than 7 days, no. (%)	42 (60.9)†	0	4 (10.0)‡	0
At least one DEC, no. (%)	29 (42.0)†	12 (26.7)	8 (20.0)‡	2 (6.7)
ETEC, no. (%)	9 (13.0)	3 (6.7)	5 (12.5)	0
DAEC, no. (%)	7 (10.1)	0	0	0
EPEC, no. (%)	6 (8.7)	4 (8.9)	2 (5.0)	0
EAEC, no. (%)	5 (7.2)	5 (11.1)	0	2 (6.7)
STEC, no. (%)	1 (1.4)	0	0	0
EIEC, no. (%)	0	0	1 (2.5)	0
DAEC + ETEC, no. (%)	1 (1.4)	0	0	0

\*DEC = diarrheagenic *E. coli*; ETEC = enterotoxigenic *E. coli*; DAEC = diffusely adherent *E. coli*; EPEC = enteropathogen *E. coli*; EAEC = enteroagregative *E. coli*; STEC = Shiga toxin producing *E. coli*; EIEC = enteroinvasive *E. coli*.

†Ten patients had HIV-negative status, 30 had unknown HIV status.

‡BMI: body mass index \* $P < 0.05$  for the comparison between HIV<sup>+</sup>D<sup>+</sup> and HIV<sup>-</sup>D<sup>+</sup>.

Ninety of 114 HIV<sup>+</sup> patients had a CD4 count measured between 90 days before and after the stool sample was taken. There were no statistical differences in the frequency of DECs between those who had  $\geq 200$  and  $< 200$  cells/mm<sup>3</sup> (Table 2).

In the HIV<sup>+</sup>D<sup>+</sup> group, 36/69 (52.2%) had persistent diarrhea (14 days or more) when they were enrolled. The frequency of having at least one DEC was higher in patients who had persistent diarrhea (50% versus 33%) (Table 3).

## DISCUSSION

We found a high frequency of DEC in HIV<sup>+</sup>D<sup>+</sup> patients but also in HIV<sup>+</sup>D<sup>-</sup> individuals using a high sensitivity and specificity method for *E. coli* pathotypes detection.<sup>3</sup> Colonization with enteric pathogens is common in other populations, such as children from developing countries.<sup>5,6</sup> A complex relationship between factors related to the host (cell immunosuppression), the bacterial agent, and the environment (high frequency of exposure to enteric pathogens caused by fecal contamination of water and food) may be responsible for the enteric pathogen colonization in HIV individuals from developing countries.

The ETEC is a well-recognized diarrheal agent among children in the developing world and in travelers,<sup>7,8</sup> but has not been frequently studied in HIV populations. In this study, ETEC was the most frequent DEC in HIV<sup>+</sup>D<sup>+</sup> and HIV<sup>-</sup>D<sup>+</sup> (~13%), but was also found in HIV<sup>+</sup>D<sup>-</sup> patients. In a previous study in Lima, Cárcamo and others<sup>9</sup> found ETEC in 4% of AIDS patients with diarrhea (but using a different technique).

The EAEC is also related to diarrheal diseases in small children and is a leading pathogen in travelers' diarrhea.<sup>8,10</sup> Previous studies have shown a strong correlation between EAEC and AIDS patients with diarrhea.<sup>1</sup> We found EAEC in HIV<sup>+</sup>D<sup>+</sup> but we also found a higher frequency of EAEC in patients without diarrhea. This rate of EAEC colonization in HIV<sup>+</sup> patients could be related to the factors described previously.

The role of DAEC in HIV-associated diarrhea has not been adequately studied. Of interest, in this study we found DAEC only in the group of HIV patients with diarrhea. There are very few studies that have looked at the prevalence of DAEC in HIV patients, therefore it is important to replicate this study to confirm this unique finding. The role of DAEC in diarrheal disease has been controversial because some studies reported similar frequency of isolation from diarrhea and control samples. However, other case-control studies have found a correlation between DAEC infection and diarrhea, especially in children  $> 12$  months of age.<sup>11,12</sup> In a recent diarrhea cohort study in infants from peri-urban communities of Lima we found that although DAEC accounted for only 5% of the cases, it was significantly associated with diarrhea in infants 6–12 months of age.<sup>13</sup> The DAEC strains are identified on the basis of their diffuse adherence pattern on cultured epithelial cells. One of the adherence factors of DAEC is the surface fimbriae that are responsible for the diffuse adherence phenotype in a prototype strain. These fimbriae are homologous with members of the Afa/Dr family of adhesins,<sup>14</sup> which are identified by hybridization to specific probes, *daaC* or *daaD*, common to operons encoding Afa/Dr adhesions. The gene used in our multiplex PCR (*daaD*) is for an initial screening of

TABLE 2

Distribution of diarrheagenic *Escherichia coli* (DEC) isolated among human immunodeficiency virus (HIV)-positive patients based on CD4 count\*

DEC type	CD4 count $\leq 200$		CD4 count $> 200$	
	With diarrhea $N = 45$ n (%)	Without diarrhea $N = 15$ n (%)	With diarrhea $N = 11$ n (%)	Without diarrhea $N = 19$ n (%)
At least one DEC	18 (40.0)	4 (26.7)	6 (54.5)	7 (36.8)
ETEC	5 (11.1)	0	1 (9.1)	3 (15.8)
DAEC	5 (11.1)	0	2 (18.2)	0
EPEC	3 (6.7)	1 (6.7)	3 (27.3)	2 (10.5)
EAEC	4 (8.8)	3 (20.0)	0	2 (10.5)
STEC	1 (2.2)	0	0	0

\*ETEC = enterotoxigenic *E. coli*; DAEC = diffusely adherent *E. coli*; EPEC = enteropathogen *E. coli*; EAEC = enteroagregative *E. coli*; STEC = Shiga toxin producing *E. coli*.

TABLE 3

Frequency of diarrheagenic *Escherichia coli* (DEC) in human immunodeficiency virus (HIV)-positive patients based on diarrheal episode duration\*

DEC type	HIV-positive	
	Diarrhea < 14 days N = 33 n (%)	Diarrhea ≥ 14 days N = 36 n (%)
At least one DEC	10 (30.3)	18 (50.0)
ETEC	3 (9.1)	6 (16.7)
DAEC	2 (6.1)	5 (13.9)
EPEC	3 (9.1)	3 (8.3)
EAEC	2 (6.1)	3 (8.3)
EAEC + ETEC	0	1 (2.7)

\*ETEC = enterotoxigenic *E. coli*; DAEC = diffusely adherent *E. coli*; EPEC = enteropathogen *E. coli*; EAEC = enteroaggregative *E. coli*.

DAED strains. Other virulence markers may be worth studying to further define more virulent strains.

The main limitation of this study is the small number of patients included in each subgroup and that the HIV status was undocumented in some control patients. Furthermore, the duration of diarrhea episodes were different in both groups being longer in HIV<sup>+</sup> patients. We conclude that different types of DEC are frequent in stools from HIV<sup>+</sup> patients with and without diarrhea regardless of the duration of diarrheal episode and immunity status. Further studies with a higher number of participants should be done to evaluate the role of the different DEC pathotypes in AIDS patients from Latin America.

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## REFERENCES

- Gassama-Sow A, Sow PS, Gueye M, Gueye-Ndiaye A, Parret JL, Mboup S, Aidara-Kane A, 2004. Characterization of pathogenic

- Escherichia coli* in human immunodeficiency virus-related diarrhea in Senegal. *J Infect Dis* 189: 75–78.
- Nataro JP, Kaper JB, 1998. Diarrheagenic *Escherichia coli*. *Clin Microbiol Rev* 11: 142–201.
- Guion CE, Ochoa TJ, Walker CM, Barletta F, Cleary TG, 2008. Detection of diarrheagenic *Escherichia coli* by use of melting-curve analysis and real-time multiplex PCR. *J Clin Microbiol* 46: 1752–1757.
- Barletta F, Ochoa TJ, Ecker L, Gil AI, Lanata CF, Cleary TG, 2009. Validation of five-colony pool analysis using multiplex real-time PCR for detection of diarrheagenic *Escherichia coli*. *J Clin Microbiol* 47: 1915–1917.
- Porat N, Levy A, Fraser D, Deckelbaum RJ, Dagan R, 1998. Prevalence of intestinal infections caused by diarrheagenic *Escherichia coli* in Bedouin infants and young children in Southern Israel. *Pediatr Infect Dis J* 17: 482–488.
- Quiroga M, Oviedo P, Chinen I, Pegels E, Husulak E, Binztein N, Rivas M, Schiavoni L, Vergara M, 2000. Asymptomatic infections by diarrheagenic *Escherichia coli* in children from Misiones, Argentina, during the first twenty months of their lives. *Rev Inst Med Trop Sao Paulo* 42: 9–15.
- Cama RI, Parashar UD, Taylor DN, Hickey T, Figueroa D, Ortega YR, Romero S, Perez J, Sterling CR, Gentsch JR, Gilman RH, Glass RI, 1999. Enteropathogens and other factors associated with severe disease in children with acute watery diarrhea in Lima, Peru. *J Infect Dis* 179: 1139–1144.
- Taylor DN, Bourgeois AL, Ericsson CD, Steffen R, Jiang ZD, Halpern J, Haake R, Dupont HL, 2006. A randomized, double-blind, multicenter study of rifaximin compared with placebo and with ciprofloxacin in the treatment of travelers' diarrhea. *Am J Trop Med Hyg* 74: 1060–1066.
- Cárcamo C, Hooton T, Wener MH, Weiss NS, Gilman R, Arevalo J, Carrasco J, Seas C, Caballero M, Holmes KK, 2005. Etiologies and manifestations of persistent diarrhea in adults with HIV-1 infection: a case-control study in Lima, Peru. *J Infect Dis* 191: 11–19.
- Huang DB, Mohanty A, DuPont HL, Okhuysen PC, Chiang T, 2006. A review of an emerging enteric pathogen enteroaggregative *Escherichia coli*. *J Med Microbiol* 55: 1303–1311.
- Spano LC, Sadovsky AD, Segui PN, Saick KW, Kitagawa SM, Pereira FE, Fagundes-Neto U, Scaletsky IC, 2008. Age-specific prevalence of diffusely adherent *Escherichia coli* in Brazilian children with acute diarrhoea. *J Med Microbiol* 57: 359–363.
- Scaletsky IC, Fabbriotti SH, Carvalho RL, Nunes CR, Maranhão HS, Morais MB, Fagundes-Neto U, 2002. Diffusely adherent *Escherichia coli* as a cause of acute diarrhea in young children in Northeast Brazil: a case-control study. *J Clin Microbiol* 40: 645–648.
- Ochoa TJ, Ecker L, Barletta F, Mispireta M, Gil AI, Contreras C, Molina M, Amemiya I, Verastegui H, Hall ER, Cleary TG, Lanata CF, 2009. Age-related susceptibility to infection with diarrheagenic *E. coli* in infants from peri-urban areas of Lima, Peru. *Clin Infect Dis* 49: 1694–1702.
- Le Bouguéne C, Servin AL, 2006. Diffusely adherent *Escherichia coli* strains expressing Afa/Dr adhesins (Afa/Dr DAEC): hitherto unrecognized pathogens. *FEMS Microbiol Lett* 256: 185–194.